

Effects of protein deficiency on testosterone levels, semen quality and testicular histology in the developing male rat

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Summary

The aim of the study was to evaluate the effects of protein deficiency on plasma testosterone levels, epididymal sperm characteristics and testicular histology in the developing male rat. Twenty four Wistar albino rats were used in the study and were fed with a diet variously containing 3 %, 10 % and 20 % protein. Plasma testosterone levels were significantly lower in rats fed 3 % protein when compared to rats fed 10 % and 20 % protein ($P<0.05$). Testicular weight in protein deficient rats (rats fed 3 % and 10 % protein) was significantly lower than that of a control group (20 % protein) ($P<0.05$). No sperm was present in the epididymal duct lumen, but germ cells were observed in some duct lumens in rats fed 3 % protein. Epididymal weight decreased significantly in rats fed 3 % protein compared to rats fed 10 % and 20 % protein ($P<0.05$). The results of this study clearly show that protein deficiency in developing male rats adversely affects gonadal endocrine function, testicular histology and spermatogenesis.

Introduction

Specific deficiencies in the intake and utilization of nutrients can adversely influence reproductive functions (McDonald, 1980). The effects of nutritional restrictions on fertility are more notable in the female than in the male. However, nutritional deficiencies do delay the onset of puberty and depress the production and quality of semen in the male. Common nutritional factors include caloric protein and vitamin deficiencies, but mineral or toxic agents may also be important (Hafez, 1987). Maturation and growth of the reproductive organs in experimental animals have been reported to be retarded by protein deficiency (Glass et al, 1979; Kalla et al, 1990; Gonzalez-Reimers et al, 1994). Previous investigations have shown that starvation and protein-calorie malnutrition cause atrophy of the reproductive system (Herbert, 1980). It has been reported that the seminiferous tubule of malnourished rats significantly decreased in diameter and in the stage of development of spermatogenesis (Guaragna et al, 1986). This is associated with changes in circulating androgen and gonadotropin levels

and subsequent disruption of spermatogenesis (Vawda and Mandlwana, 1990).

Nutrition affects the endocrine rather than the spermatogenic function of the testis (Hafez, 1987). Neurons controlling release of gonadotropin-release hormone are extremely sensitive to changes in nutritional state (Robinson, 1990). The hypothalamo-hypophyseal-gonadal axis is impaired when the consumption of protein and calories is decreased. Serum luteinising hormone, follicle stimulating hormone, prolactin and testosterone are lower in malnourished rats at all ages (Herbert, 1980).

The young and growing animal is much more susceptible to nutritional stress than the mature animal, and malnutrition constitutes a greater stress to spermatogenesis in prepubertal males (McDonald, 1980). This study was undertaken to evaluate the effects of protein deficiency on plasma testosterone levels, epididymal sperm characteristics and testicular histology in developing male rats.

Materials and Methods

Animals

Twenty-four male Wistar Albino rats (28-32 days of age) were selected as the experimental subjects.

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They were acclimatized for 7 d with laboratory conditions at 22-25 °C with a 12 h light/dark cycle and were then randomly divided into three groups of eight animals each. The eight rats in each group were assigned to two cages of four rats and fed with a diet containing 3 %, 10 % and 20 % protein (control). The compositions of these experimental diets are shown in Table 1. They, were analyzed for dry matter (DM), ash, crude protein (CP), crude fiber (CF), and ether extract (EE; *AOAC, 1980*). Percentages of organic matter (OM) and nitrogen free

extract (NFE) were calculated. The rats were fed ad libitum and had free access to clean tap water throughout the experiment. Three of the rats in the 3 % protein group died at day 45 of the experiment. The remaining rats were sacrificed by decapitation under ether anaesthesia two months after initiation of the experiment. The rats received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health.

Table 1. Composition of experimental diets

Ingredients	20 % Group	10 % Group	3 % Group
	% of Total diet		
Casein	25.0	12.5	3.0
Corn oil	7.5	7.5	7.5
Vitamin-mineral mix	7.0	7.0	7.0
Starch	57.5	70.0	79.5
Peanut hull	3.0	3.0	3.0
	Chemical composition of diet		
DM	95.25	94.98	93.91
Ash, % DM	12.59	12.27	12.52
OM, % DM	87.41	87.73	87.48
CP, % DM	18.33	9.18	2.96
EE, % DM	7.98	7.14	8.56
CF, % DM	0.36	0.26	0.34
NFE, % DM	60.74	71.15	75.62

Blood and sperm sampling

Blood samples were collected from the neck. Plasma was obtained after centrifugation (1500 g, 20 min, 4 °C) and stored at - 20 °C for testosterone assays. Rat sperm samples were collected from cauda epididymidis using the aspiration methods described by Slott et al. (1991). After collecting blood samples, testes were quickly removed. The epididymis was clamped with a hemostat approximately at the corpus-cauda junction and the hemostat angled so that the vas deferens was clamped as well. A small cut was made with a scalpel blade into the engorged tubules of the distal cauda epididymis. The epididymal content was aspirated by a capillary tube connected to a 2 ml syringe containing 1 ml of prewarmed (37 °C) phosphate-buffered saline (PBS), pH 7.3. The epididymal fluid with medium was put into a 2 ml tube containing 0.5 ml PBS. The

sample was placed in a drying oven at 37 °C for 5 min to allow the spermatozoa to disperse into the medium. This sample was used for determination of both motility and number of abnormal spermatozoa. For estimating epididymal sperm concentrations, the sperm suspension obtained by cutting the other cauda epididymidis was directly drawn into a red blood cell pipette.

Examination of epididymal sperm

The percentage of motile sperm was determined using a phase contrast microscope equipped with an electrically heated (37 °C) stage. A few drops of the sperm suspension were examined under the 40 x objective of the microscope and motile sperm counted in 10 random fields and expressed as a percentage of the total number present (*Vawda and Mandlwana, 1990*).

In order to investigate sperm morphology, a drop of sperm suspension was incubated for 5 min at 37 °C in 0.5 ml formol-citrate solution (37 % formaldehyde, 4 ml; 2.9 % sodium-citrate, 96 ml). Then, a few drops of this suspension was mounted on a glass slide and examined under an immersion objective. A total of 200 spermatozoa were analysed per animal. The spermatozoa were categorized as normal or abnormal according to the methods described by Spinks et al. (1997).

Epididymal sperm counts were determined with a modification of the methods described by Kempinas and Lamano-Carvalho (1988). Briefly, the sperm suspension was drawn into red blood cell pipette up to the 0.1 line and diluted to 1:1000 with Hayem solution (NaSO₄, 5 gr; NaCl, 1gr; HgCl₂, 0.5 gr; 200 ml redistilled water). In order to disperse the spermatozoa, the pipette was shaken for about 5 min and the spermatozoa counted on a Thoma slide.

Histology

Testes and epididymis were carefully removed, weighed, and transferred into neutral buffered formalin. After fixing, the tissues were routinely processed for the microtome, sectioned at a thickness of 6 µm, and stained with Crossman's Triple stain

(Culling et al, 1985). Histological measurements were made on a light microscope fitted with a stage-ocular micrometer combination at 10x magnification.

Radioimmunoassay for plasma testosterone

Circulating testosterone levels were measured by radioimmunoassay using a commercially available assay kit (Immunotech, France). The sensitivity was 0.1 ng/ml.

Statistical analysis

The results were analyzed by one-way ANOVA. The mean treatment differences were determined by Bonferroni's t-test with a level of statistical significance of 5 % (SPSS, 1999).

Results

The means and SEM plasma testosterone concentrations, number of epididymal sperm, percentage of motility and abnormal spermatozoa, testes and epididymis weights and seminiferous tubule diameters are presented in Table 2. No spermatozoa were observed within fluid obtained from cauda epididymis of rats fed 3 % protein, except one of the animals had about 20 abnormal spermatozoa.

Table 2. The means and SEM of plasma testosterone concentrations, spermatologic features, testicular and epididymal weights in control and protein deficient rats

	20 % Group (n=8)	10 % Group (n=8)	3 % Group (n=5)
Testis weight (g)	1,31 0.04 ^a	1.08 0.03 ^b	0.28 0.04 ^c
Epididymis weight (g)	0.53 0.02 ^a	0.61 0.03 ^a	0.20 0.03 ^b
Seminiferous tubule diameter (µm)	166.4 5.43 ^a	172.0 7.9 ^a	102.0 5.3 ^b
Testosterone levels (ng/ml)	2.23 0.14 ^a	2.16 0.11 ^a	1.44 0.16 ^b
Motility (%)	62.25 5.6	58.38 3.1	Not determined
Concentration (x10 ⁷ /ml)	164.4 10.8	139.5 8.0	Not determined
Abnormal spermatozoon number	24.18 3.13	19.88 1.94	Not determined

a,b,c; mean values in same rows with no common superscripts differ significantly (p<0.05).

The percentage of motile sperm was not significantly different between rats fed 10 % and 20 % protein. The concentration of spermatozoa found in

the cauda epididymis tended to be lower in rats fed 10 % protein than those fed 20 % protein (P<0.09). Although the percentage of morphologically abnor-

mal spermatozoa was higher in rats fed 20 % protein than those fed 10 % protein, this difference was not significant. Plasma testosterone levels were significantly lower in rats fed 3 % protein compared to rats fed 10 % and 20 % protein ($P < 0.05$).

Histological examination of the testes indicated that the seminiferous tubules were adversely affected only in the rats fed 3 % protein (Table 2; Figs. 1,2). No or little spermatozoon was present in the lumen of the seminiferous tubules of those rats. Testicular weight in the protein deficient rats (3 % and 10 % protein) was significantly lower than that of control group (20 % protein) ($P < 0.05$; Table 2). There was no spermatozoon present in the epididymal duct lumen, but germ cells were observed in some duct lumen of rats fed 3 % protein (Fig. 3). Epididymal weight significantly decreased in rats fed 3 % protein compared to other groups (10 % and 20 % protein) (Table 2).

Discussion

Despite the ability of a mature male to maintain sperm and testosterone secretion under low levels of nutrition, the young male shows retarded sexual development and delayed puberty. This is due to suppression of endocrine activity of the testes and consequently, to the retardation of growth and secretory function of the male reproductive organs. The results of the present study indicated that dietary protein deficiency had a detrimental effect on testicular function depending on the degree of protein deficiency in the developing male rats. Vawda and Mandlwana (1990) reported that the feeding rats on diets containing 10 % protein appears to have little or no adverse effect on testicular function, but protein deficiencies (0 % and 5 %) have adverse effects on epididymal sperm concentrations, motility and morphology in sexually mature rats. The absence of adequate protein has far reaching effects on spermatogenesis and epididymal sperm maturation (Deo, 1978). Vawda and Mandlwana (1990) suggested that the Sertoli cells control and nurture spermatogenesis, via a host of protein factors. In this study, epididymal sperm concentration, and the percentage of motile and abnormal spermatozoa were not different in rats fed 10 % protein compared to the control group. The azoospermia and necrospermia found in rats fed 3 %

protein can be attributed to lowered testosterone levels, inhibited spermatogenesis, underdeveloped testes and delayed puberty.

Plasma testosterone levels were significantly lower in rats fed 3 % protein compared to rats fed 10 % protein and control diets ($P < 0.05$). Vawda and Mandlwana (1990) also reported that plasma androgen levels were lower in the 0 % and 5 % protein-deficient groups in sexually mature rats. Male rats exposed to a low-protein diet from 20 to 80 days of age have consistently similar serum gonadotropin and testosterone values to those found in prepubertal animals. Protein-deficient rats were capable of producing mature sperm, although the timing for the onset of spermatogenesis was delayed by 2.5-3 weeks (Herbert, 1979). Jean-Faucher et al. (1982) also determined that there was a delay in the maximal production of testosterone by the testis in male mice underfed from 20 to 60 days and was 50 % lower than that of controls. However, the mice had reached puberty and spermatogenesis was preserved. In the current study, plasma testosterone levels of rats fed 3 % protein were similar to previous studies, but no mature sperm was observed in the cauda epididymis of rats in the current study, which was an indicator of not having reached puberty. This difference is probably due to the different protein quantity in the diet and the duration of the experiment in these studies.

Glass et al. (1984) reported that testicular weight was lower in rats fed a low protein (9 %) diet than that of a control group early in the experiment (80 days). In undernourished rats, body and testes weights were significantly lower (Herbert, 1980; Jean-Faucher et al, 1982). Vawda and Mandlwana (1990) also reported that testicular weight in protein-deficient rats was significantly lower than that of a control group. These studies support the results of our study in which we observed no or few spermatozoa in the seminiferous tubules, and germ cells (not spermatozoon) in some epididymal ducts of rats fed 3 % protein. (Fig. 3). The epididymal ducts of rats fed 10 % and 20 % protein were filled with spermatozoa. Herbert (1980) reported that on light - microscopic examination of the testes, normal maturation of the germ cells failed in the rats fed a low protein diet.

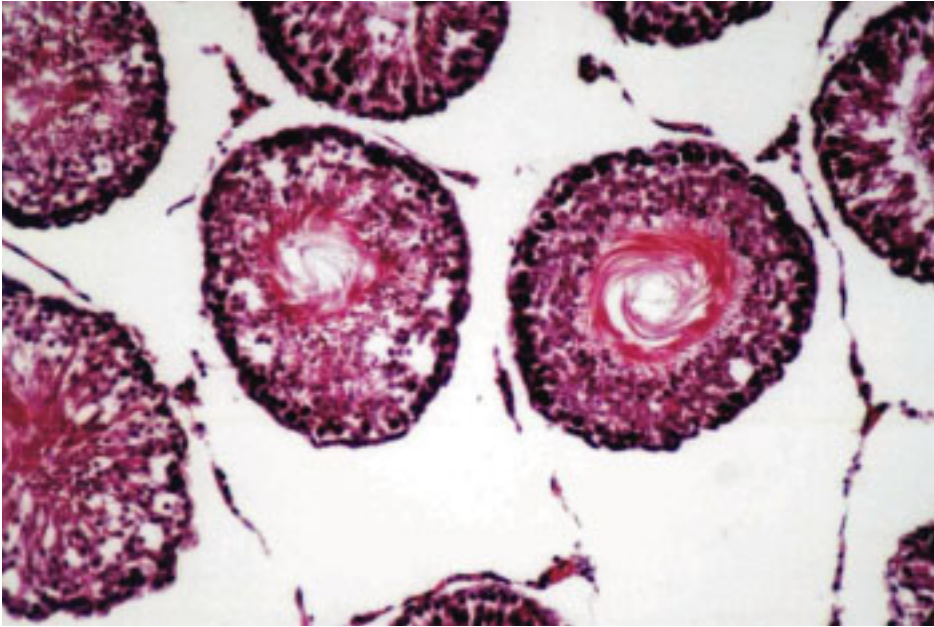


Figure 1. Photomicrograph of testes in control group. Triple staining, x300.

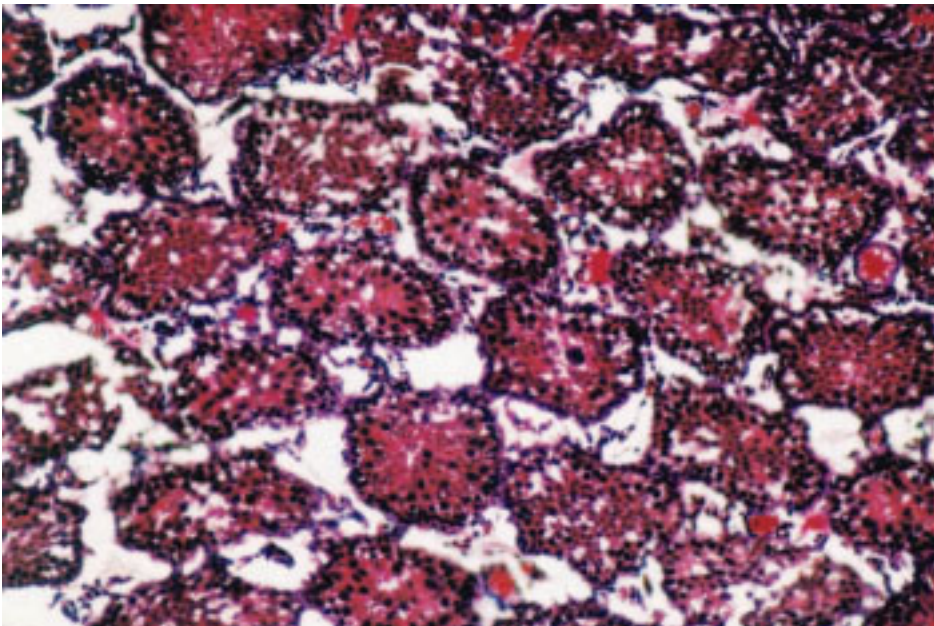


Figure 2. Photomicrograph of testes in 3 % protein group. Triple staining, x300.

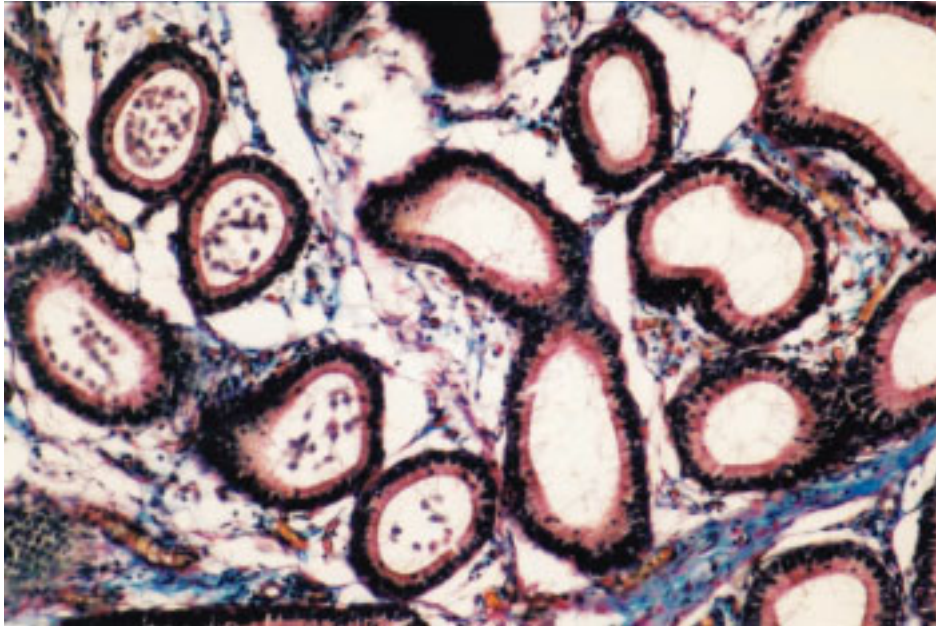


Figure 3. Photomicrograph of epididymis in 3 % protein group. Triple staining, x300.

Protein deficiency and lowered level of testosterone may result in an impairment of gonadal organ function and retardation or inhibition of spermatogenesis.

Protein deficiency plays a major role in tubular atrophy (Gonzalez-Reimers et al, 1994). Vawda and Mandlwana (1990) reported that while seminiferous tubule diameters in rats fed 0 % and 5 % protein were significantly lower than that of the control group, seminiferous tubule diameters of rats fed 10 % protein were similar to that of the control group. Glass et al. (1979) also reported that seminiferous tubule diameters were normal in the low protein (9 %) group. These findings support the results of the current study. However, Guaragna et al. (1986) reported that a decrease in diameter of seminiferous tubules was observed in rats receiving an 8 % protein diet for 20 days. This difference is probably due to differences in experimental period.

The results of the current study clearly showed that protein deficiency plays an important role in gonadal endocrine function, testicular histology and spermatogenesis. Testicular functions were detri-

mentally affected in the 3 % protein-deficient group, but no adverse effects were observed in the 10 % protein-deficient group. Therefore, it was concluded that developing rats receiving 10 % protein are able to maintain normal testicular function.

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